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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

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Diagnostic and therapeutic use of an ATP-binding cassette gene and protein for neurodegenerative diseases

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DIAGNOSTIC AND THERAPEUTIC USE OF AN ATP-BINDING CASSETTE GENE AND PROTEIN FOR NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating, and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social, and economic burden. AD is the most common age-related neurodegenerative condition affecting about 10% of the population over 65 years of age and up to 45% over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles. AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-192).

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon4 allele of apolipoprotein E (ApoE). Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP), presenilin-1, and presenilin-2, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

A number of studies on the etiology of AD have pointed to a role of dysregulated cholesterol levels to be associated with an increased risk of developing the disease. This puts AD in the context of other neuro-degenerative disorders caused by unique genetic defects in either cholesterol biosynthesis (e.g., Smith-Lemli-Opitz syndrome and desmosterolosis) or intracellular transport of cholesterol (Niemann-Pick Type C disease). However, while the latter syndroms and diseases are autosomal recessive diseases caused by defects in one particular gene only (for review, Nwokoro et al., Mol. Genet. Metab. 2001, 74: 105-119), the late onset form of AD is considered to be a complex and genetically heterogeneous disorder caused by widespread polymorphisms with low penetrance but high prevalence (Tanzi and Bertram, Neuron 2001, 32: 181-184).
Regarding AD, an early hallmark finding was that a particular allele of the ApoE apolipoprotein, namely the epsilon 4 allele, is associated with an increased risk and early onset of AD (Corder et al., Science 1993, 261: 921-

923). ApoE is a lipoprotein found at high levels in both, plasma and brain. In peripheral tissues (i.e., outside the liver) it is involved in the degradation of cholesterol- and triglyceride-rich low density lipoprotein (LDL) particles. ApoE can bind to the LDL receptor, and it is the affinity of the binding to this receptor that determines the role of the different allelic ApoE forms in arteriosclerosis and AD, i.e. the epsilon 2 allele has a certain cholesterol lowering effect in plasma and is associated with a decreased risk of AD while the epsilon 4 allele is associated with elevated plasma cholesterol levels and an increased risk of AD (for review, Golde and Eckman, Drug Discovery Today 2001, 6: 1049-1055). However, >85% of the variability of the plasma cholesterol is independent of the carrier's ApoE genotype (Breslow, Annu. Rev. Genet. 2000, 34: 233-254). Furthermore, it is widely agreed that the vast majority of the brain's cholesterol is synthesized in situ (Dietschy and Turley, Curr. Opin. Lipidol. 2001, 12: 105-112) and, therefore, ApoE's role in the brain is likely restricted to regional cholesterol redistribution and a potential direct effect on aggregation of APP-derived A beta 40/A beta 42 peptides (Mahley, Science 1988, 240: 622-631; Castano et al., Biochem. J. 1995, 306: 599-604).

Recently, it has been recognized that cholesterol levels in the membrane and/or the cellular ratio of free cholesterol to cholesterol esters would directly impact on membrane fluidity and, indirectly, on the processing of the amyloid precursor protein by the counteracting alpha- and beta-/gamma-secretases. Cholesterol depletion from cells was demonstrated to impair the amyloidogenic beta-/gamma-secretase-dependent pathway while favoring the non-amyloidogenic alpha-secretase-dependent pathway (Golde and Eckman, Drug Discovery Today 2001, 6: 1049-1055; Simons et al., Neurology 2001, 57: 1089-1093; Wolozin, Proc. Natl. Acad. Sci. U.S.A. 2001, 98: 5371-5373; Puglielli et al., Nat. Cell Biol. 2001, 3: 905-912). Together, the picture that emerges is that an overall lowering of cellular cholesterol levels in cells is of benefit with respect to developing AD.This is further and strongly supported by epidemiologic studies using drugs that block de novo synthesis of cholesterol in the whole body, including the brain. Such treatment markedly reduced the incidence of AD in the investigated populations (Wolozin et al.,

Arch. Neurol. 2000, 57: 1439-1443; Jick et al., Lancet 2000, 356: 1627-1631). The same beneficial impact of cholesterol lowering drugs was observed in experimental animal models of AD (Refolo et al., Neurobiol. Dis. 2000, 7: 321-331; Fassbender et al., Proc. Natl. Acad. Sci U.S.A. 2001, 98: 5856-5861).

Given that the brain's cholesterol is synthesized in situ, there is a need for a homeostatic mechanism of reverse transport of cholesterol from the brain to the liver for bile acid production and excretion. The current opinion is that a major pathway for reverse transport involves the enzymatic formation of 24-hydroxycholesterol („cerebrosterol"), its diffusion across cellular membranes and the blood-brain-barrier, and the subsequent transport via circulation to the liver for extraction, modification, and excretion (Dietschy and Turley, Curr. Opin. Lipidol. 2001, 12: 105-112). Interestingly, CYP46, the enzyme that catalyses the formation of 24-hydroxycholesterol, is almost exclusively found in brain tissue, thus corroborating the idea of a specialized homeostatic function of this pathway (Lütjohann et al., Proc. Natl. Acad. Sci. U.S.A. 1996, 93: 9799-9804; Lund et al., Proc. Natl. Acad. Sci. U.S.A. 1999, 96: 7238-7243). In fact, plasma levels of 24-hydroxycholesterol have been suggested as stage markers of neurological diseases including AD (Papassotiropoulos et al., NeuroReport 2000, 11: 1959-62; Bretillon et al., Neurosci. Lett. 2000, 293: 87-90; Bogdanovic et al., Neurosci. Lett. 2001, 314: 45-48).

The instant invention discloses the differential expression of ABCA1, a member of the large and well investigated family of ATP-binding cassette transporters, in different regions of the AD brain but not in the brains of healthy age-matched control individuals. ABCA1 is a 2261 amino acid protein encoded by the ABCA1 gene on chromosome 9 (Santamarina-Fojo et al., Proc. Natl. Acad. Sci. U.S.A. 2000, 97: 7987-7992; GenBank accession number AF275948). ATP-binding cassette transporters make up a family of membrane-embedded transport proteins that utilize the energy of ATP-hydrolysis to accomplish vectorial trans-membrane transport of cargo molecules like ions, metabolites, protein fragments and others. Well-known members of this family are the MDR1, TAP and CFTR transporters, having roles in multidrug resistance, peptide presentation by MHC class I molecules,

and cystic fibrosis, respectively (for recent review, Dean et al., Genome Res. 2001, 11: 1156-1166).

ABCA1 has been shown to act as an energy-dependent reverse cholesterol and phospholipid transporter in macrophages (for review, Oram and Lawn, J. Lipid. Res. 2001, 42: 1173-1179). ABCA1 mRNA was found in the following tissues: liver, intestine, placenta, adipose, adrenal glands, macrophages, and spleen (Langmann et al., Biochem. Biophys. Res. Comm. 1999, 257: 29-33; Chawla et al., Science 2001, 294: 1866-1870). Mutations in the ABCA1 gene lead to Tangier disease and familial high density lipoprotein deficiency (Brooks-Wilson et al., Nat. Genet. 1999, 22: 336-345; Bodzioch et al., Nat. Genet. 1999, 22: 347-351; Rust et al., Nat. Genet. 1999, 22: 352-355; Oram and Lawn, J. Lipid. Res. 2001, 42: 1173-1179). The potential of ABCA1 as a diagnostic marker and therapeutic target for arteriosclerosis, coronary artery disease, cardiovascular diseases and lipid disorders in general has been recognized (WO 00/55318, WO 00/78971, WO 00/78972, WO 00/18912, WO 01/15676, WO 01/70810). However, to date no experiments have been described that demonstrate a relationship between the dysregulation of ABCA1 gene expression and the pathology of neurodegenerative diseases, in particular AD. Such a link, as disclosed in the present invention, offers new ways, inter alia, for the diagnosis and treatment of said diseases.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a

translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The term 'AD' shall mean Alzheimer's disease.

Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia,

progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, ischemic stroke, age-related macular degeneration, and narcolepsy.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of the ABCA1 gene, and/or of (ii) a translation product of the ABCA1 gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of the ABCA1 gene, and/or of (ii) a translation product of the ABCA1 gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of the ABCA1 gene, and/or of (ii) a translation product of the ABCA1 gene, and/or of (iii) a fragment or derivative of said transcription or translation

product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment, said subjects suffer from Alzheimer's disease.

The present invention discloses the differential expression and regulation of the ABCA1 gene in specific brain regions of AD patients. Consequently, the ABCA1 gene and its corresponding transcription and/or translation products may have a causative role in the regional selective neuronal degeneration typically observed in AD. Alternatively, ABCA1 may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular AD. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is particularly preferred that said sample to be analyzed and determined is selected from the group consisting of a brain tissue or other tissues, organs or body cells. The sample can also consist of cerebrospinal fluid or other body fluids including saliva, urine, blood, serum plasma, or nasal mucosa.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of the ABCA1 gene, and/or of (ii) a translation product of the ABCA1 gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an increase or decrease in a transcription product of the ABCA1 gene and/or a translation product of the ABCA1 gene in a sample cell, or tissue, or body fluid from said subject relative to a reference

value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly AD.

In preferred embodiments, measurement of the level of transcription products of the ABCA1 gene is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based micro-array technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000).

Furthermore, a level and/or activity of a translation product of the ABCA1 gene and/or fragment of said translation product, can be detected using an immunoassay, an activity assay, and/or binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots, and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999). All these detection techniques may also be employed in the format of micro-arrays, protein-arrays, or protein-chip based technologies.

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of the ABCA1 gene, and/or of (ii) a translation product of the ABCA1 gene, and/or of (iii) a fragment or derivative

of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular AD, in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:
(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the ABCA1 gene (ii) reagents that selectively detect a translation product of the ABCA1 gene; and
(b) an instruction for diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop such a disease by
- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the ABCA1 gene, in a sample from said subject; and
- diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of said subject to develop such a disease,
wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular AD. Consequently, the kit, according

to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular AD, in a subject, as well as monitoring success or failure of therapeutic treatment for such a disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) the ABCA1 gene, and/or (ii) a transcription product of the ABCA1 gene, and/or (iii) a translation product of the ABCA1 gene, and/or (iv) a fragment or derivative of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation product of a gene coding for ABCA1, or a fragment, or derivative, or a variant thereof.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents, detergents, polymers,

enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against transcripts of a gene coding for ABCA1. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy.

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene

encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection, or liposomal mediated transfection.

In preferred embodiments, said agent for treating and preventing a neurodegenerative disease, in particular AD, is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and

differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an AD-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the ABCA1 gene, and/or (ii) a transcription product of the ABCA1 gene and/or (iii) a translation product of the ABCA1 gene, and/or (iv) a fragment or derivative of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the ABCA1 gene, and/or (ii) a transcription product of the ABCA1 gene, and/or (iii) a translation product of the ABCA1 gene, and/or (iv) a fragment or derivative of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the ABCA1 gene,

and/or (ii) a transcription product of the ABCA1 gene and/or (iii) a translation product of the ABCA1 gene, and/or (iv) a fragment or derivative of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular AD.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for ABCA1, or a fragment thereof, or a derivative thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular AD. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular AD.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the ABCA1 gene, and/or (ii) a transcription product of the ABCA1 gene, and/or (iii) a translation product of the ABCA1 gene, and/or (iv) a fragment or derivative of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the ABCA1 gene, and/or (ii) a transcription product of the ABCA1 gene, and/or (iii) a translation product of the ABCA1 gene, and/or (iv) a fragment or derivative of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses a gene coding for ABCA1, or a fragment thereof, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native ABCA1 gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and a translation product of the gene coding for ABCA1, or a fragment or derivative thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of said ABCA1 translation product, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding fluorescently labelled ligand to said containers, and (iv) incubating said ABCA1 translation product, or said fragment or derivative thereof, and said compound or plurality of compounds, and said fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said ABCA1 translation product, or with said fragment or derivative thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said ABCA1 translation product, or said fragment or derivative thereof. It might be preferred to reconstitute said ABCA1 translation product or fragment or derivative thereof into artificial liposomes to generate the corresponding proteoliposomes to determine the inhibition of binding between a ligand and said ABCA1 translation product. Methods of reconstitution of ATP binding cassette transporters from detergent into liposomes are known to those

skilled in the art (Hagmann et al., Eur. J. Biochem. 1999, 265: 281-289; Ahn et al., J. Biol. Chem. 2000, 275: 20399-20405). Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to an ABCA1 translation product, or a fragment or derivative thereof.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of the gene coding for ABCA1 by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to a translation product of the gene coding for ABCA1, or to a fragment or derivative thereof. Said screening assay comprises (i) adding a liquid suspension of said ABCA1 translation product, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said ABCA1 translation product, or said fragment or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds, and (iv) measuring the amounts of fluorescence associated with said ABCA1 translation product, or with said fragment or derivative thereof, and (v) determining the degree of binding by one or more of said compounds to said ABCA1 translation product, or said fragment or derivative thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Also in this type of

assay it might be preferred to reconstitute an ABCA1 translation product or fragment or derivative thereof into artificial liposomes as described in the present invention . Said assay methods may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to an ABCA1 translation product.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of the ABCA1 gene by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the ABCA1 gene or a fragment thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized,

or single chain antibodies, as well as fragments thereof. Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods involving detecting translation products of the ABCA1 gene.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, the pathological state relates to a neurodegenerative disease, in particular to AD. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in US patent 6150173.

Other features and advantages of the invention will be apparent form the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in AD. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in AD (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in AD. Brain tissues from the frontal cortex (F) and the temporal cortex (T) of AD patients and healthy, age-matched

control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 demonstrates the differential expression of the ABCA1 gene in AD brain tissues by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and temporal cortex (T) of AD patients (Fig 2A) and of a healthy, age-matched control individuals (Fig 2B) was performed by the LightCycler rapid thermal cycling technique. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figure depicts the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of the ABCA1 cDNA from both the frontal and temporal cortices of a normal control individual during the exponential phase of the reaction are juxtaposed (Fig 2B, arrow), whereas in AD (Fig 2A, arrows), there is a significant separation of the corresponding curves, indicating a differential expression of the ABCA1 gene in the two analyzed brain regions.

Figure 3 charts the schematic alignment of the ABCA1 primer sequences to the nucleotide sequence of the human ABCA1 gene (GenBank accession number AF275948); for simplicity, only exon 1 and exon 50 are sketched; polyA: polyadenylation site; primers are indicated by arrows and positional numbering.

Figure 4 outlines the exact nucleotide sequence alignment of the ABCA1 primer pair to the nucleotide sequence of the human ABCA1 gene (GenBank accession number AF275948).

Table 1 lists the gene expression levels in the temporal cortex relative to the frontal cortex for the ABCA1 gene in seven AD patients (1.18 to 2.99 fold) and five healthy, age-matched control individuals (0.81 to 1.49 fold).

EXAMPLE I:

(i) Brain tissue dissection from patients with AD:

Brain tissues from AD patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Fig. 1) and stored at –80°C until RNA extractions were performed.

(ii) Isolation of total mRNA:

Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were used to generate a melting curve with the LightCycler technology as described in the manufacturer's protocol (Roche).

(iii) Quantitative RT-PCR analysis:

The expression levels of the human ABCA1 gene in temporal cortex versus frontal cortex were analyzed using the LightCycler technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by

using a kinetic, rather than an endpoint readout. The ratio of ABCA1 cDNA in temporal cortex versus frontal cortex was determined (relative quantification). First, a standard curve was generated to determine the efficiency of the PCR with specific primers for human ABCA1 (5'-TGTTGCATCCCCCTTAGAATGT-3' and 5'-GAGGGCCAATGATGAACAAAG-3'). PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 79°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 114 bp for ABCA1 was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTT-TGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'-TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel

analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for ABCA1 and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortex and temporal cortex were analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10 \wedge ((C_t \text{ value} - \text{intercept}) / \text{slope}) \quad [\text{ng total brain cDNA}]$$

The values for frontal and temporal cortex cDNAs of ABCA1 cDNA were normalized to cyclophilin B and the ratio was calculated according to formula:

$$\text{Ratio} = \frac{\text{ABCA1 temporal [ng]} / \text{cyclophilin B temporal [ng]}}{\text{ABCA1 frontal [ng]} / \text{cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from

one gene to another gene remained constant in different runs, it was possible to normalize the values for ABCA1 to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of one such quantitative RT-PCR analysis for the ABCA1 gene are shown in Figure 2.

CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:
determining a level and/or an activity of
(i) a transcription product of the ABCA1 gene, and/or
(ii) a translation product of the ABCA1 gene, and/or
(iii) a fragment or derivative of said transcription or translation product
in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. A method of monitoring the progression of a neurodegenerative disease in a subject, comprising:
determining a level and/or an activity of
(i) a transcription product of the ABCA1 gene, and/or
(ii) a translation product of the ABCA1 gene, and/or
(iii) a fragment or derivative of said transcription or translation product
in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby monitoring the progression of said neurodegenerative disease in said subject.

3. A method of evaluating a treatment for a neurodegenerative disease, comprising:
determining a level and/or an activity of
(i) a transcription product of the ABCA1 gene, and/or
(ii) a translation product of the ABCA1 gene, and/or
(iii) a fragment or derivative of said transcription or translation product
in a sample from a subject being treated for said disease and comparing said level and/or said activity to a reference value representing a known disease or

health status, thereby evaluating said treatment for said neurodegenerative disease.

4. The method according to any of claims 1 to 3 wherein said neurodegenerative disease is Alzheimer's disease.

5. The method according to any of claims 1 to 4 wherein said sample is a cell, or a tissue, or an organ, or a body fluid, in particular cerebrospinal fluid or blood.

6. The method according to any of claims 1 to 5 wherein said reference value is that of a level and/or an activity of

(i) a transcription product of the ABCA1 gene, and/or

(ii) a translation product of the ABCA1 gene, and/or

(iii) a fragment or derivative of said transcription or translation product

in a sample from a subject not suffering from said neurodegenerative disease.

7. The method according to any of claims 1 to 6 wherein an increase or decrease in a transcription product of the gene coding for ABCA1 and/or a translation product of the gene coding for ABCA1 in a cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

8. The method according to any of claims 1 to 7, further comprising comparing a level and/or an activity of

(i) a transcription product of the ABCA1 gene, and/or

(ii) a translation product of the ABCA1 gene, and/or

(iii) a fragment or derivative of said transcription or translation product

in a series of samples taken from said subject over a period of time.

9. The method according to claim 8 wherein said subject receives a treatment prior to one or more of said sample gatherings.

10. The method according to claim 9 wherein said level and/or activity is determined before and after said treatment of said subject.

11. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the ABCA1 gene and (ii) reagents that selectively detect a translation product of the ABCA1 gene; and

(b) an instruction for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by (i) detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the ABCA1 gene, in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status indicates a diagnosis or prognosis of a neurodegenerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease.

12. A method of treating or preventing a neurodegenerative disease, in particular Alzheimer's disease, in a subject comprising administering to said subject in a therapeutically or prophylactically effective amount an agent or agents which directly or indirectly affect an activity and/or a level of

(i) the ABCA1 gene, and/or
(ii) a transcription product of the ABCA1 gene, and/or
(iii) a translation product of the ABCA1 gene, and/or
(iv) a fragment or derivative of (i) to (iii).

13. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of
(I) the ABCA1 gene, and/or
(ii) a transcription product of the ABCA1 gene, and/or
(iii) a translation product of the ABCA1 gene, and/or
(iv) a fragment or derivative of (i) to (iii).

14. A recombinant, non-human animal comprising a non-native gene sequence coding for ABCA1 or a fragment thereof, or a derivative thereof, said animal being obtainable by:
(i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and
(ii) introducing said targeting construct into a stem cell of a non-human animal, and
(iii) introducing said non-human animal stem cell into a non-human embryo, and
(iv) transplanting said embryo into a pseudopregnant non-human animal, and
(v) allowing said embryo to develop to term, and
(vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and
(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

15. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the ABCA1 gene, and/or

(ii) a transcription product of the ABCA1 gene, and/or

(iii) a translation product of the ABCA1 gene, and/or

(iv) a fragment or derivative of (i) to (iii),

said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

(d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

16. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the ABCA1 gene, and/or

(ii) a transcription product of the ABCA1 gene, and/or

(iii) a translation product of the ABCA1 gene, and/or

(v) a fragment or derivative of (i) to (iii),

said method comprising:

(a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;

(d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

17. The method according to claim 16 wherein said test animal and/or said control animal is a recombinant animal which expresses a gene coding for ABCA1, or a fragment thereof, or a derivative thereof, under the control of a transcriptional control element which is not the native ABCA1 gene transcriptional control element.

18. An assay for testing a compound, preferably for screening a plurality of compounds for inhibition of binding between a ligand and an ABCA1 translation product, or a fragment or derivative thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said ABCA1 translation product, or a fragment or derivative thereof, to a plurality of containers;

(ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers;

(iii) adding fluorescently labelled ligand to said containers;

(iv) incubating said ABCA1 translation product, or said fragment or derivative thereof, and said compound or compounds, and said fluorescently labelled ligand;

(v) measuring amounts of fluorescence associated with said ABCA1 translation product, or with said fragment or derivative thereof; and

(vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said ABCA1 translation product, or said fragment or derivative thereof.

19. An assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to an ABCA1 translation product, or to a fragment or derivative thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said ABCA1 translation product, or a fragment or derivative thereof, to a plurality of containers;

(ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers;

(iii) incubating said ABCA1 translation product, or said fragment or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds;

(iv) measuring amounts of fluorescence associated with said ABCA1 translation product, or with said fragment or derivative thereof; and

(v) determining the degree of binding by one or more of said compounds to said ABCA1 translation product, or said fragment or derivative thereof.

20. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the gene coding for ABCA1, or a fragment or derivative thereof, for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell, and wherein said pathological state relates to a neurodegenerative disease, in particular Alzheimer's disease.

Figure 1: Identification of genes involved in Alzheimer's Disease pathology

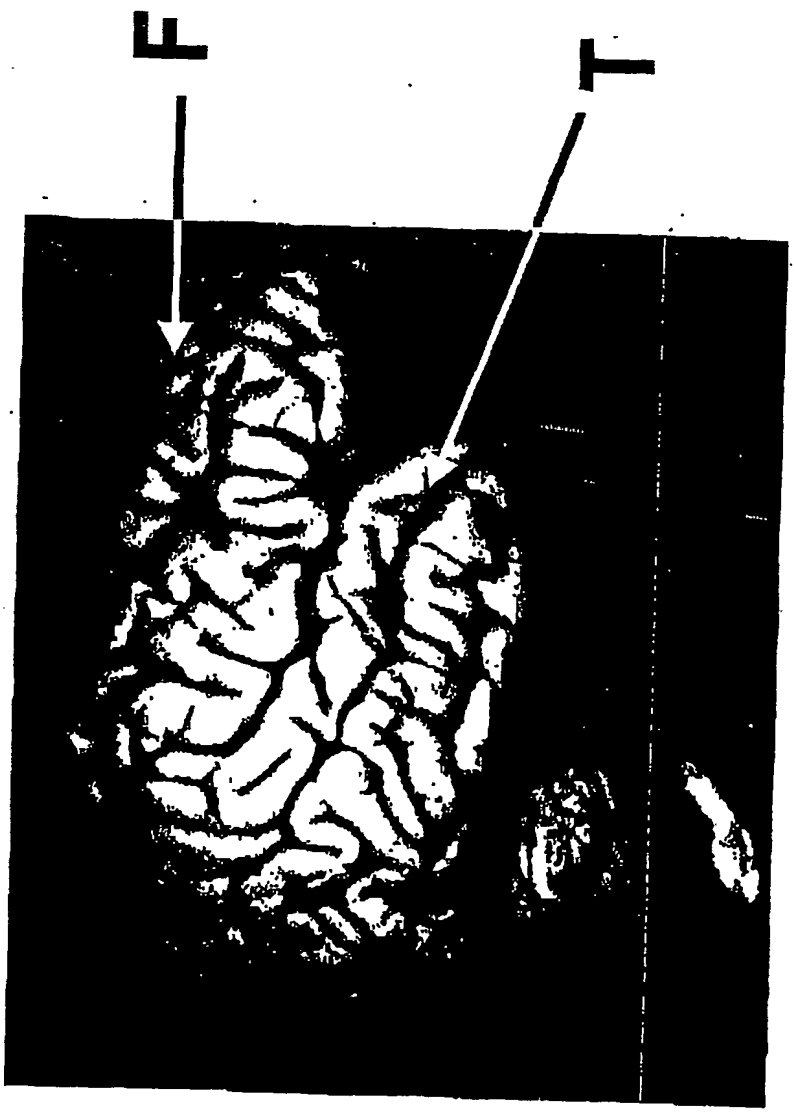

Figure 2: Differential expression of ABCA1 as determined by RT-PCR analysis

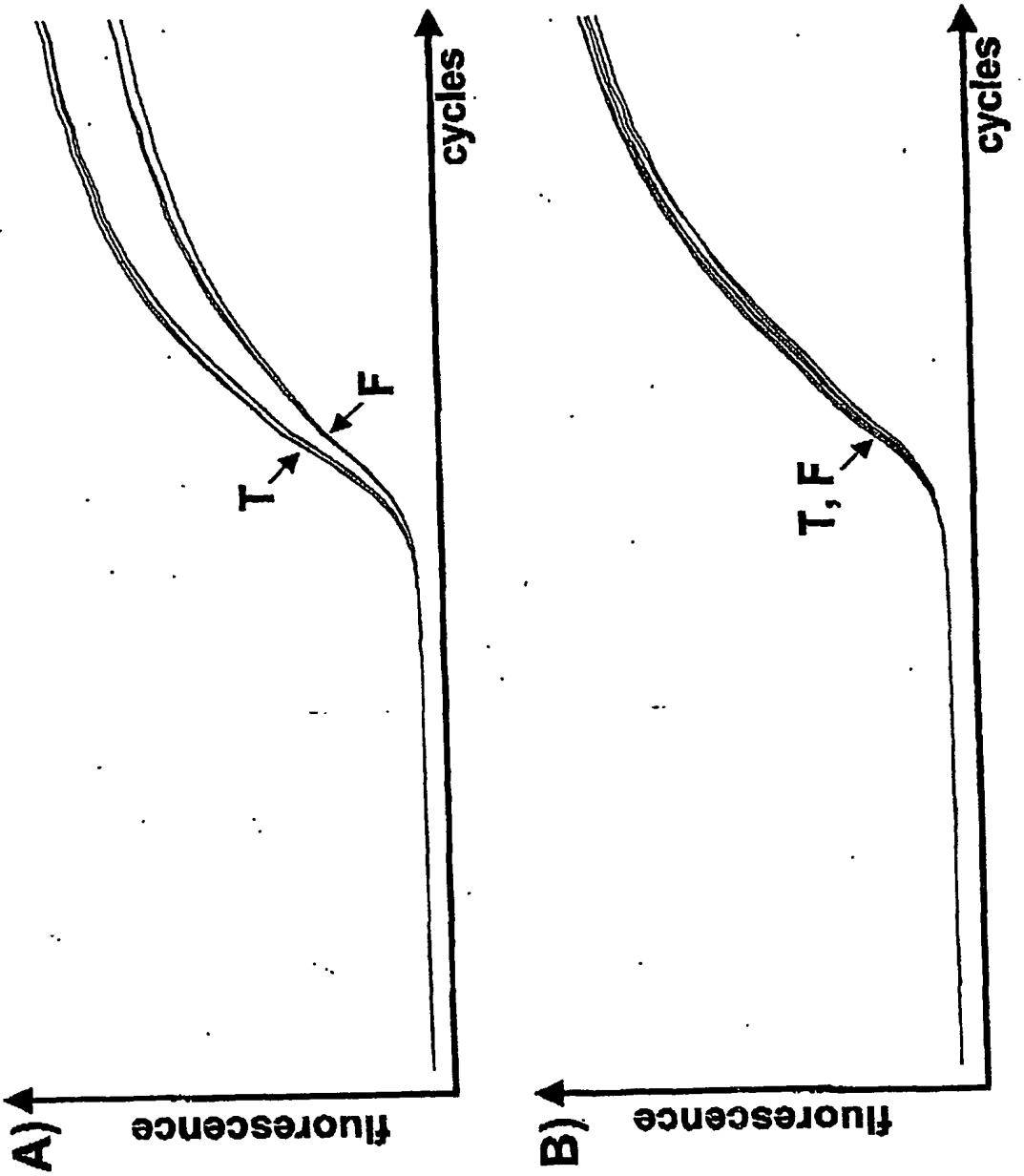

Figure 3: Schematic alignment of ABCA1 RT-PCR primers with human ABCA1 cDNA (GenBank accession number: AF275948)

Figure 4: Alignment of ABCA1 RT-PCR primers with ABCA1 cDNA, GenBank accession number AF275948

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     1 TGTTGCATCCCCCTTAGAATGT 22
       ||||||||||||||||||||||
147679 TGTTGCATCCCCCTTAGAATGT 147700

     1 CTTTGTTCATCATTGGCCCTC  21
       |||||||||||||||||||||
147772 CTTTGTTCATCATTGGCCCTC  147792
```

Table 1: Differential expression of the ABCA1 gene in the AD brain

sample	Δfold (temporal / frontal cortex)
control 1	1.04
control 2	0.85
control 3	0.81
control 4	1.49
control 5	1.40
patient 1	1.18
patient 2	2.48
patient 3	1.55
patient 4	1.80
patient 5	1.45
patient 6	2.35
patient 7	2.99

SUMMARY

The present invention discloses the differential expression of the ABCA1 gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or for determining whether a subject is at increased risk of developing such a disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using the ABCA1 gene and its corresponding gene products. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.